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CROCHEMICAL STUDIES ON WHEAT SEEDLINGS IN RELATION TO THEIR REACTION TO FOOT-ROT PATHOGENES

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University of Alberta
April, 1933.

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MICROCHEMICAL STUDIES ON WHEAT SEEDLINGS IN RELATION TO THEIR REACTION TO FOOT-ROT PATHOGENES

Lawrence Edmund Tyner
Department of Field Crops

A THESIS

submitted to the University of Alberta
in partial fulfilment of the requirements for
the degree of
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MICROCHEMICAL STUDIES ON WHEAT SEEDLINGS IN RELATION TO THEIR REACTION TO FOOT-ROT PATHOGENES

Lawrence Edmund Tyner

INTRODUCTION

Of late years a number of investigators have been approaching the question of disease susceptibility and resistance in plants from the point of view of the carbohydrate metabolism. It is well known that pathogenic fungi show degrees of preference for the various carbohydrate tissues of their hosts, and that these preferences depend upon the enzyme complement of each fungus. Careful studies on culture media have shown the carbohydrate range for many common fungous parasites and saprophytes.

Dickson and Holbert (7) have reported that the difference in susceptibility of wheat and corn seedlings to "blight" caused by Gibberella saubinetii under favorable and unfavorable temperature conditions is due to the actual sugars and polysaccharides in relation to their percentage fractions of the total carbohydrates. They concluded that the pentosans and pentosan complexes constitute the most favorable food supply for the organism. High temperatures in the case of the wheat seedlings favored an increase in the percentage fractions of such polysaccharides. This condition was found to be reversed in the case of corn seedlings, which showed

resistance to blight at the higher temperatures, and this was coupled with a low pentosan content.

In western Canada, Ophiobolus graminis, a fungus which is probably indigenous, is our most destructive foot-rotting pathogene. It was thought advisable to study the reaction of wheat seedlings to this pathogene under controlled environmental conditions which might be expected to modify the carbohydrate metabolism of the host. Similar studies were also made with other common foot-rotting pathogenes, namely: Helminthosporium sativum, and Fusarium culmorum. The study is an extension of the work done in this laboratory by Mr. H. T. Robertson (22) upon the histological phenomena associated with the invasion of wheat tissues by O. graminis.

The primary concern of this investigation, however, is to establish some foundation for the hypothesis that foot-rotting fungi exhibit preferences for the different carbohydrates of the wheat plant. Particular attention is given to the role of the pentosans and pentosan complexes found in wheat seedlings. Chemical and microchemical tests have been carried out in an endeavour to find a reliable quantitative measure of the pentosan content of plant tissues grown under various environmental conditions. It is believed that such measurements might be taken as indices of the responses of the wheat plant to the foot-rotting pathogenes under different environments.

LITERATURE REVIEW

1. Pentosans as Fungus Food.

Hawkins (11) in 1915, working with the fungus Glomerella cingulata, discovered that by inoculating half an apple with this organism and leaving the remainder uninoculated, more furfural producing substances were yielded by the inoculated rotted half than by the sound control. From cultural experiments on a synthetic medium of NH_4NO_3, KH_2PO_4, $MgSO_4$ with the addition of hexoses, pentoses or pentosans, he concluded that the organism developed better on the pentose or pentosan medium. He also demonstrated the ability of an extract of the organism to hydrolyse xylan into xylose.

Campbell (2) in studying the white rots of woods showed that various fungi exhibit preferences for the specific pentosan complexes; thus Armillaria mellea attacked the non-cellulose pentosans and avoided the cellulose pentosans, whereas Polyporus hispidus acted in an exactly opposite manner.

Norman (19,20) in discussing the nature and quantity of the furfural yielding substances of straws points out that in the decomposition of straw the cellulose disappears most quickly, hence is the most important item of food. In an extension of the above work in 1930 Norman employed the following organisms in the decomposition of cereal straws: Aspergillus flaviceps, A. fumigatus, A. nidulans, A. niger, A. terreus, Trichoderma spp., Phoma spp., Actinomyces spp. He tested the availability of various nitrogen

and carbon compounds to these organisms. Particular attention was directed to those sugars naturally occurring in that class of substances known as the hemicelluloses (hexoses, uronic acids and pentose units). The pentose sugars, though readily available, did not appear so suitable for fungous food as their sterically related hexoses. The maximum period of heat evolution was found to correspond with the most rapid loss of hemicelluloses. This class of saccharides decompose very rapidly during the first few days because they are encrusting substances of the cell walls and must be removed before the wall can be attacked.

Dickson (6) in his conclusions from experiments on the host-parasite complex of corn and wheat infested with Giberella saubinetii states that pentose yielding substances such as pectin and xylan are excellent fungous foods and produce five times more vegetative growth of the fungus than do the hexoses, and other soluble sugars. He summarizes as follows:-

> "Any metabolic balance which results in the formation of easily hydrolysable cell walls and the accumulation of pectic and other pentose substances predisposes the seedlings to fungus attack."

Young (25) studying the penetration phenomena of Alternaria and Diplodia spp. observed a definite change in the chemical composition of cell walls at the point of the fungus penetration. These changes were no doubt due to enzyme activity, the pectins of the middle lamella being dissolved (5). However, whether the products resulting from pectin dissolution are preferred as food by the

advancing hyphae is doubtful.

Lüdtke (15) in experiments with Ophiobolus graminis and Fusarium culmorum showed by microchemical tests upon the cell walls of invaded wheat plants, that these pathogenes were concerned only incidentally with the cell walls during penetration. He considered it probable that the greater susceptibility of young plants to these parasites was due to the action of the parasite's enzymes upon the polymerous carbohydrates of the host, the success of the enzyme activity being dependent upon the coincidental development of host and parasite.

2. Relationships of Temperature to Carbohydrate Metabolism.

Dickson (6) analysed winter wheat grown at soil temperatures ranging from 16° - 36° and found that the carbohydrate content was greatest in the plants at 20° C., decreasing in amount at the higher temperatures. The nitrogen content was least at the greatest concentration of the carbohydrates and increased as the carbohydrate fraction decreased. He attributed temperature effects in disease development primarily to changes in the host. Thus he observed that corn plants "blight" at temperatures of 12° - 20° C., at which temperatures wheat plants are relatively free from injury by the organism Gibberella saubinetii. On the other hand, he observed that at soil temperatures of 24° - 30° C. corn plants remain healthy, whereas wheat is severely affected.

Jones, Johnson and Dickson (13) from a study of the relation of soil temperature to plant disease conclude: That at temperatures above 20° C. the plumule of the young wheat plant pushes through

the coleoptile before emerging from the soil, whereas at temperatures of 16° or less the plumule does not push through until after emergence, hence embryonic tissues are preserved from infection during the early stages of growth. Adverse soil temperatures, according to these writers, create an unbalanced metabolism in the seedling which leads to the accumulation of pentosan-like substances, providing good fungous food. Plants with a well balanced metabolism on the other hand contain chiefly substances which are relatively poor in fungous food values.

3. Relationships of Light to Carbohydrate Metabolism.

Garner, Bacon, and Allard (9) found that the duration of the daily light period may definitely control the carbohydrate metabolism and other parallel processes of fundamental importance in plant development. The period of illumination may determine not only the quantity of carbohydrates produced but also controls the utilization of these substances. The light period in some way influences acidity relations, the form of carbohydrate present, and also probably the water content of the tissue.

Shirley (23) studied the influence of light intensity and quality upon the following plant species: buckwheat, *Fagopyrum esculentum*; sunflower, *Helianthus annuus*; wandering Jew, *Tradescantia iluminensis*; tomato, *Lycopersicum esculentum*; tobacco, *Nicotiana tabacum*. He found: (1) that at low light intensities the dry weight produced by the plants studied was almost directly proportional to the light intensity received up to about 20 per cent of full sunlight; (2) that the ratio of dry weight of roots to dry weight of

shoots, the percent of dry matter in tops, the density of growth, the strength of stem, and the leaf thickness, all increased with increasing light intensity.

Reid (21) continuing the work of Shirley made comparisons of plants grown in light and in darkness. He found that the weight of shoots in proportion to the weight of roots of seedlings grown in the light is lower than that of seedlings grown in darkness. The shift in proportion is brought about especially by the shorter stems, and in most cases by the much larger root systems of the seedlings grown in light. Light favored the assimilation of nitrates, especially by high protein seedlings with relatively low carbon reserves. Light was found to favor secondary thickening in stems and roots and the deposition of strengthening materials in the cell walls.

Arthur, Guthrie and Durrell (1) found that small grains such as barley and spring wheat in contrast to potatoes, will grow well and yield well at a high temperature (25° C.) if given additional light and CO_2. The production of these grains is not favored by low temperatures when day length is long and the CO_2 supply abundant. The weight per plant of barley increased with the day length up to a 19 hour day. The total carbohydrates also increased and nitrogen decreased. Analyses were made on plants 30-48 days old.

The conclusions mentioned by Arthur certainly do not substantiate the theories of Jones, Johnson and Dickson regarding the effects of temperature upon the metabolism of the seedling.

Nightingale (18) investigated the chemical composition of

plants in relation to photoperiodic changes. He used tomato plants grown for several weeks under normal greenhouse light conditions in summer. The plants were divided into two groups, one group being harvested and dried and the other group being placed in a dark chamber for an additional eleven days. A hemicellulose analysis of the stems from each treatment revealed 12.16 per cent in the first harvested fraction and 7.96 per cent in the latter. Thus, apparently the synthesis of this class of polysaccharides not only ceased in the darkness as one might expect, but heavy demands were made upon the available supply.

Clements (3) studied the influence of photoperiodism on the carbohydrate complex in Solanum tuberosum var. Russet Rural, Soja max var. Ito San, and Helianthus annuus. He observed large and frequent variations in the hemicellulose content of leaves at different times of the day and at different seasons. Hexoses and pentoses were the sugars first formed by the plant. The hemicelluloses, besides serving as building substances and reserve food, were observed to be of importance in the degree of drought resistance of the plants.

EXPERIMENTAL METHODS

Qualitative Tests for Pentosans.

At the outset a number of procedures for staining pentosans in plant tissues were tried:

1. (a) Sections of seminal roots, secondary roots, and the subcoronal internodes of seedlings of various wheat varieties were

fixed in form-acetic-alcohol, washed, dehydrated in a butyl alcohol series, and embedded in paraffin. Sections 10 u in thickness were cut and the slides treated in a xylol-alcohol series before staining in aniline acetate (16).

(b) Benzidine hydrochloride in alcoholic solution (26).

No appreciable staining of tissues occurred upon treatment with either of these reagents.

2. Free-hand sections of the sub-coronal internodes of wheat and corn seedlings were cut about 0.5 mm. thick. These sections, when treated directly with either of the reagents (a) or (b) above, showed the presence of pentosans in all the lignified elements of the vascular system. Using aniline acetate, these tissues stained yellow, while with the benzidine hydrochloride solution they stained a dark pink. Mounts were made with Canada balsam, but it was found that the colors faded out from the sections in a few weeks' time. Consequently, if any permanent record were to be obtained from this phase of the investigation it was thought necessary to find a staining procedure which would achieve a more permanent staining reaction.

3. Dr. J. G. Dickson (6) kindly forwarded an outline of the procedure employed in an analogous study. In respect to that part of his outline dealing with pentosan staining his method consists of the following procedure:

"Cut free-hand sections of tissue and transfer to 10 cc. of 0.5 per cent ammonium oxalate solution. Heat on water bath at 95° C. for two hours to remove pectic acid. Extract the sections from the ammonium oxalate solution with 4.0 per cent sulphuric acid on the water bath at 95° C. for 1 - 2 hours. Wash and transfer to Ruthenium red 1 - 10,000. Stain 12 hours in darkness. Transfer to safranin 1 - 1,000 aqueous, 30 - 40 minutes. Transfer to Sudan IV.

saturated solution in 70 per cent alcohol with 3 - 4 drops conc. sulphuric acid per 50 cc. of solution. Let stand 6 - 12 hours."

This treatment showed pentosan units to be present in the epidermis, the endodermis, the pericycle and all the lignified elements of the vascular system. No staining was demonstrated in the cortex nor in the pith. Upon mounting the sections in glycerine jelly and covering it was found that the colors did not fade over a period of some months if the slides were stored in the dark. Ruthenium red ($Ru_2 (OH)_2 Cl_4$) is, unfortunately, a very light sensitive reagent and care must be taken in the staining procedure and after mounting to avoid any undue exposure to light. The safranin and Sudan IV probably help in fixing it however.

It was hoped that the above technique would afford a reliable quantitative index of the pentosan content of plant sections by means of variations in staining intensity. However, no reliance could be placed in the method on a color intensity basis, since all tissue elements carrying pentose units stained in the same manner regardless of the quantity of pentosans present. Any value inherent in the technique is dependent upon the following features:

(1) The pentosan yielding areas are orientated.

(2) There is an increased facility in making quantitative measurements under the microscope of the pentosan bearing cells.

(3) A rapid method of roughly estimating the pentosan content by the extent of staining in a particular section is available.

Various applications of the method will be recorded under the experiments on light and temperature which follow.

Quantitative Estimation of Pentosans.

The method employed was that of Krober (10) as adopted by the Association of Official Agricultural Chemists.

The material to be analysed was washed thoroughly and cut into one inch portions. These were dried at 70° C. for 48 hours, then ground to a fine powder in a meat chopper. A sample of 1.5 grams was weighed out accurately and placed in a 500 cc. distilling flask connected to a water cooled condenser with a 300 mm. jacket. A quantity of 100 cc. of 12 per cent hydrochloric acid was added through a graduated dropping funnel and heat applied to the bottom of the flask. The heat was so regulated that approximately 30 cc. of distillate passed over in ten minutes. After this quantity had distilled the distillation was discontinued and 30 cc. more of HCl were added and the distillation resumed. This process was continued until 475 cc. had passed over. A measured quantity (0.25 gm.) of phloroglucinol (Merck C.P.), dissolved in 25 cc. of the acid, was added slowly with vigorous stirring and the distillate let stand for 36 hours, when the precipitate of phloroglucide was filtered off through tared Gooch crucibles fitted with asbestos mats. These were dried at 97° for four hours, and weighed before cooling.

The pentosan was computed from the formula: P = (Weight of precipitate + .0052 gm.) X 0.8866.

This method depends upon the fact that pentosans are converted into furfural in a weak hydrolytic solution; this reacts with the phloroglucinol to form an insoluble phloroglucide

$$(C_5H_{10}O_5)_n - 3H_2O = (C_4H_3O - CHO)_n$$

Pentosan Furfural

$$(C_4H_3O - CHO) + C_6H_6O_3 = C_{11}H_6O_3 + 2H_2O$$

Furfural Phloro-glucinol Phloroglucide.

Several factors militated against the application of this method as a reliable quantitative measure of pentosans in plant tissue. Some of these factors are difficult if not impossible to overcome, as they arise from the biologic nature of the material tested. Thus it is thought that the difficulty of obtaining homogeneous samples was an important factor. Furthermore, it has not yet been possible to guard against slight errors arising from the technique of the method used such as unequal precipitation rates of the phloroglucide, and the destruction of some of the furfuraldehyde by failure to pass off as fast as it is generated. However, although a certain degree of experimental error is unavoidable, quite good checking results can be obtained by using this method.

EXPERIMENTS AND RESULTS

Effects of Temperature on Carbohydrate Metabolism.

Experiment 1.

During the winter of 1931 - 32 preliminary experiments upon temperature effects were conducted in the greenhouse. Forty 6 inch pots of 3:1 soil and sand mixture were sterilized and planted to Marquis wheat (Elite selection U. of A.). Ten pots were placed on a bench in one section of the greenhouse in full sunlight, which was supplemented by artificial light for 7 hours per day. The average soil temperature was 12° C. Ten pots were placed below the

bench and remained there for the 30-day period of growth, at an average soil temperature of 15° C. Ten pots were placed on a bench in another section of the greenhouse at an average soil temperature of 17° C. The remaining ten pots were placed below this bench where the soil temperature was approximately 20° C.

After cutting free-hand sections from the sub-coronal internodes of plants grown under the different conditions, and staining for pentosans by the Ruthenium red method, a microscopic examination showed:

1. That lignification, and consequently pentosan staining, was much less apparent in the sections of plants grown under the benches than in those grown in the more intense light.
2. That there was more advanced lignification of tissues and more intense staining of sections from plants grown at 17° in the light than in those at 12° in the light.
3. That staining was more pronounced in sections of the material grown at 20° in the shade than in sections from the material grown at 15° in the shade.

In this preliminary study, it was evident that differences in pentosan content, due to these environmental factors, were at least demonstrable microchemically. It appeared that light was probably more important than temperature as an influencing factor.

Experiment 2.

A further test of the effects of temperature was made during July, 1932, when sections were cut from Marquis wheat seedlings grown in the constant temperature tanks at temperatures of approximately (16°,

21°, 26° 31°) and made available through the courtesy of Dr. A. W. Henry. Examinations of the stained sections showed that the most complete and general staining occurred in the sections from material grown at 26° C. Sections from the material grown at 16° and at 21° were weakly lignified, and showed less staining. The 31° material showed a much stronger staining reaction. Some notes were made from this examination which are given in table 1.

Table 1. Effect of temperature on the extent and distribution of lignin in the cell walls of Marquis wheat seedlings.

Soil temperature in degrees Centigrade	Average thickness in microns of vessel walls	Average thickness in microns of inner tangential walls of the endodermis	Average number of stained cells per field (X400)
16.8	3.0	2.5	115
21.2	2.9	2.7	138
26.5	3.2	2.8	160
31.4	3.2	2.7	145

A better idea of the differences in lignification and staining will be gained from an inspection of the two representative microphotographs shown in figures 1 and 2.

Experiment 3.

As a more reliable method of judging the percentage of pentosans to be found in wheat seedlings grown under varied temperature conditions it was decided to grow further material and analyse it chemically. Accordingly, Marquis wheat seed was planted in unsterilized soil in 6 inch pots in November, and grown for a period of 32

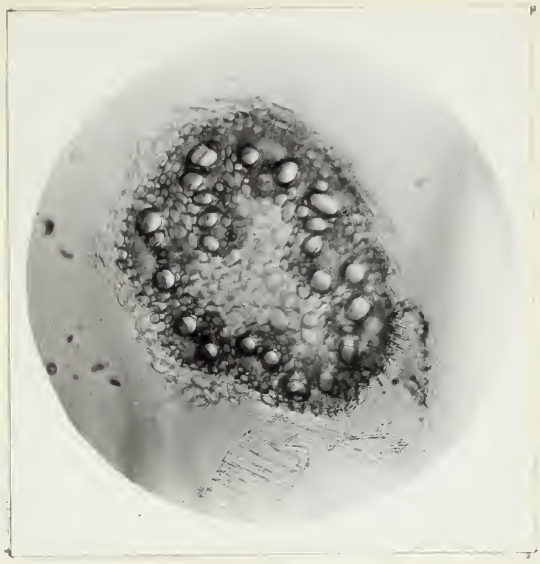

Figure 1. Free-hand section of the sub-coronal internode of a 23-day Marquis wheat seedling grown at 16° C. June - July. Staining shows pentosan-bearing tissue and the extent of lignification. X100.

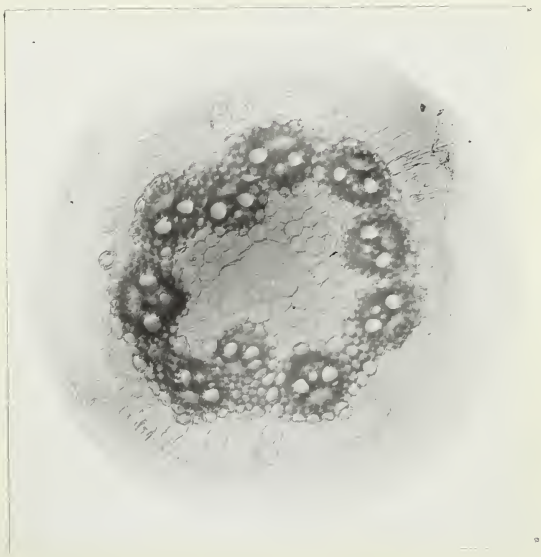

Figure 2. Free-hand section of the sub-coronal internode of a 23-day Marquis wheat seedling grown at 31° C. June - July. Staining shows pentosan-bearing tissue and the extent of lignification. X100.

days in the constant temperature tanks. The soil temperatures at which the seedlings were grown were approximately 16°, 21.5°, 25°, and 30.5°, as average readings over this period.

The material was harvested on December 11, dried and ground. The results of the analyses made are summarized in table 2.

Table 2. Results of chemical analyses of Marquis wheat seedlings grown at different temperatures.

Soil temperature in degrees C.	Total dry weight of plants in gms.	Weight of phloroglucide yielded in gms.		Percentage of pentosans	Average percentage of pentosans
16.0	6.09	1.	0.1080	6.74	
		2.	0.1070	6.63	
					6.68
21.5	5.80	1.	0.1046	6.49	
		2.	0.0986	6.12	
					6.30
26.0	4.90	1.	0.0904	5.65	
		2.	0.0846	5.31	
					5.48
30.5	4.16	1.	0.1014	6.30	
		2.	0.0936	5.84	
					6.07

These pentosan percentages are rather low and the duplicates do not check well, hence the differences cannot be considered significant. The fact that the differences shown among the four treatments do not agree with those in the previous table might be explained by the factor of difference in length of daylight periods prevailing. Here it might be apropos to again refer to the conclusion of Arthur et al (1) that the growth of wheat plants is not favored at low temperatures when the days are long and the CO_2 supply abundant.

If the pentosan percentages are not significant, yet the dry

weights of the different treatments would seem to bear out Dickson's (6) statement that the metabolism of the plant is unbalanced at higher growth temperatures. However, his statement that there is a greater concentration of pentosans at higher growth temperatures is not borne out, at least not under the short day lengths of the above experiment.

Moreover, in this connection, it is of interest to note that Henry (12) demonstrated a greater incidence of infection by Ophiobolus graminis on wheat seedlings grown at temperatures of 13° to 18° than at higher temperatures in unsterilized soil. If we positively correlate his findings with the pentosan content of the plants grown under the low temperature conditions in the present study it further complicates an understanding of the statements of Dickson.

Effects of Light upon Carbohydrate Metabolism.

Experiment 4.

The preliminary work was done in the greenhouse as indicated in the section on temperature effects. Following these, more attention was directed toward securing controlled conditions of illumination. Accordingly, four small cages were constructed and covered with white cheesecloth. Half the cages were covered with two layers, and the remaining half with six layers. Twenty-four 6 inch pots of soil planted to Marquis wheat were used in the experiment, twelve being placed outdoors and twelve within the greenhouse. Each group of twelve were subdivided as follows:

4 pots under the six layer cage;

4 pots under the two layer cage;

4 pots uncovered.

Table 3 expressed the results of observations upon 10 stained sections for each treatment after 17 days growth.

Table 3. Effect of light on the extent and distribution of lignin in the cell walls of Marquis wheat seedlings after 17 days growth.

Treatment	Average thickness in microns of vessel walls	Average thickness in microns of inner tangential walls of endodermis	Average number of stained cells per field (X400)
Greenhouse			
Unshaded	2.2	3.0	170
Medium light	2.0	1.5	115
Low light	1.5	0.5	80
Outside			
Unshaded	2.25	3.0	150
Medium light	2.15	2.0	130
Low light	2.0	1.75	75

It was noted that the ratio of the thickness of endodermal walls to vessel walls increased as the illumination increased. Moreover, as the age of the seedling increased there was manifested a tendency for the ratio of the thickness of the endodermal walls to vessel walls to become greater than one. This will be seen upon an examination of table 4.

Those plants growing outside the greenhouse developed a more extensive degree of lignification of the endodermal cells. Not only were these cells more sturdy, but the same was found to apply to the cells of the vascular ring linking the bundles. In this experiment again, no control of temperature was attempted, and there can be no

assurance that the temperature differences were not influential.

Further notes were taken on material from the above experiment which was grown an additional 18 days, or for a total of 35 days during May and June. These results appear in table 4.

Table 4. Effect of light on the extent and distribution of lignin in the cell walls of Marquis wheat seedlings after 35 days growth.

Treatment	Average thickness in microns of vessel walls	Average thickness in microns of inner tangential walls of endodermis	Average number of stained cells per field (X400)
Greenhouse			
Uncovered	2.4	3.0	165
Medium light	2.4	2.5	140
low light	2.0	1.5	112
Outside			
Uncovered	2.5	3.3	170
Medium light	2.4	2.8	130
Low light	1.8	1.8	90

Experiment 5.

An attempt was next made to grow Marquis seedlings under more measurable conditions of illumination, and it was thought best to exclude daylight altogether. One of the refrigeration rooms was subdivided into three equal compartments by screens. Mazda lamps were suspended midway to the floor in each compartment, and thermographs were standardized, and one placed in each compartment in positions as closely as possible alike in respect to the suspended lamps, being sufficiently remote to prevent the influence of direct radiation from the lamps. The total watts of the lamps were 800, 400 and 200 for the three light sources. Flats planted to Marquis

wheat were placed in each compartment and the light intensity in lumens or foot candle power at the soil surface determined with a photometer.

800 watts illumination yielded 160 lumens;

400 " " " 100 " ;

200 " " " 38 " .

All lights were put on at 4 p.m. and switched off at 8 a.m., making a light period of 16 hours per day. An electric fan was turned on and off with the lights.

Some temperature data for the experiment follows:

Table 5. Soil and air temperature ranges under conditions of 200, 400, and 800 watt illuminations in refrigeration room.

Treatment	Maximum soil temperature F.	Minimum soil temperature F.	Maximum air temperature F.	Minimum air temperature F.
800 Watt chamber	61	52	66	58
400 Watt chamber	57	52	63	58
200 Watt chamber	54	52	63	58

Plantings were made on June 1, and the material was taken up on June 15. Samples of each treatment were used in an histological study and sections were cut and treated in the manner previously described. From an examination of the stained and mounted sections it was found that lignification in the 200 and 400 watt material had not progressed sufficiently far to allow of any conclusions regarding the staining reaction, since the sections collapsed and

masked the results. The sections from the 800 watt material showed that lignification had occurred to an extent sufficient to lend a degree of rigidity to the tissues, capable of withstanding the rather vigorous manipulation necessary in this free-hand sectioning technique. Pentosan staining was well advanced in these sections.

It may be noted that Reid (21) found that the process of secondary thickening of stems and roots of many seedlings and the deposition of materials in the cell walls were very much inhibited in darkness.

The remaining plant material gathered from the experiment was cut, dried, powdered and analysed as described under Krober's method of pentosan analysis. The results of the analysis are given in table 6.

Table 6. Results of chemical analysis of Marquis wheat seedlings grown under illumination of 800, 400 and 200 watts.

Treatment	Dry weight of sample in gms.	Weight of phloroglucide precipitated in gms.	Percent of pentosans
800 Watts	3.236	0.6395	17.58
400 "	3.643	0.5833	14.23
200 "	2.363	0.1688	6.48

Although insufficient of the dry plant material was available from the experiment to run duplicate analyses, nevertheless, the rather wide differences in the pentosan content indicated that the light treatments were of decided influence.

Experiment 6.

It was thought advisable to grow a larger bulk of material in

a larger space, sacrificing the rather absolute light control obtainable in the dark room in favor of a more regular temperature and a greater growing area. Accordingly the method of Experiment 4 was extended and a whole greenhouse bench was used, divided into three portions, each to accomodate 15 flats about one foot square. A framework of lumber was erected over two sections of the bench to a height of three feet above the surface. One section was fully covered with six layers of 28 - 28 mesh cheesecloth, the second with two layers, and the third section remained uncovered. The cheesecloth covering was brought down only to within two inches of the surface of the bench to favor ventilation of the chamber.

The soil temperatures were taken at frequent intervals during the experiment from August 1 to September 2, and it was found that the temperature of the soil in all three compartments did not vary at all during cloudy days and after sunset. On sunny days a variation of 1.5° C. was recorded as between the six layer cheesecloth chamber and the uncovered section, it being lower in the covered section. This control was secured by the adjustment of the greenhouse curtains.

On September 2 four flats from each chamber were harvested, a portion being used for histological examination and the remainder washed and dried at once.

The microscopic examination of the stained sections showed fundamentally the same degrees of difference between material grown under different light intensities. It will be noted from Table 7 that the magnitudes of the measurements have decreased from those obtained on material grown earlier in the summer. This is quite to

be expected, however, due to the changing light intensity with the season's advance.

Davis and Hoagland (4), experimenting upon the extent of wheat growth in different seasons, found variations in dry weight of wheat plants grown at different times of the year from 15.2 to 97.5 grams per 100 tops.

In table 7 is shown a comparative tabular treatment of the measurements taken on the material examined September 2, and on part of the same experiment allowed to grow until September 22.

Table 7. Effects of light on the extent and distribution of lignin in the cell walls of Marquis wheat seedlings.

Treatment	Average thickness in microns of vessel walls	Average thickness in microns of inner tangential walls of endodermis	Average number of stained cells per field (X400)
30-day plants			
Uncovered	2.1	1.3	110
Medium light	1.9	1.0	88
Low light	1.65	0.8	16
50-day plants			
Uncovered	2.5	2.8	174
Medium light	2.1	2.0	133
Low light	2.1	1.5	84

Photographs were taken of sections from the material gathered on September 22, after 50 days growth, and these will illustrate the progress of lignification and the degree of staining of the cells under the different treatments. See figures 3, 4 and 5.

Analyses of the dried plant material were made upon the sample harvested September 22. The former sample was divided into two

Figure 3. Free-hand section of the sub-coronal internode of a 50-day Marquis wheat seedling grown under normal illumination, August - September. Staining shows the pentosan-bearing tissue. X100.

Figure 4. Free-hand section of the sub-coronal internode of a 50-day Marquis wheat seedling grown under medium illumination, August - September. Staining shows the pentosan-bearing tissue. X100.

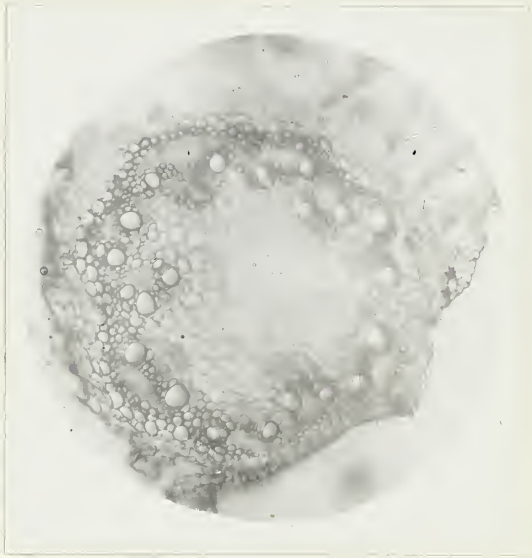

Figure 5. Free-hand section of the sub-coronal internode of a 50-day Marquis wheat seedling grown under low illumination August - September. Staining shows the pentosan bearing tissue. X100.

parts, the upper leafy portions and the lower portions, including the root system plus about two inches of the shoot. Because of the impossibility of absolutely separating roots from shoots and securing an adequate sample of the roots this practice of dividing the plants and running separate analyses has not been followed in subsequent studies.

Data on the analyses appear in table 8.

Table 8. Results of chemical analyses of Marquis wheat seedlings grown under varied light conditions.

Treatment	Weight of sample in gms.	Weight of precipitate of phloroglucide in gms.	Percentage of pentosans	Average pentosan content
30-day plants				
Low light				
Tops 1.	2.0	0.1581	7.31	
2.	2.0	0.1020	4.76	
Roots 1.	1.5	0.1534	9.37	
2.	1.41	0.1370	8.94	
				7.59%
Medium light				
Tops.1.	1.5	0.1430	8.76	
2.	1.5	0.1547	9.45	
Roots 1.	1.5	0.1802	10.96	
2.	1.5	0.1845	11.21	
				10.09%
Natural light				
Tops 1.	1.5	0.1721	10.48	
2.	1.5	0.1872	11.37	
Roots 1.	1.5	0.1812	11.02	
2.	1.5	0.1982	12.02	
				11.22%
50-day plants Roots and tops mixed				
Low light 1.	1.5	0.1308	8.04	
2.	1.5	0.1390	8.52	
				8.28%
Medium lightl.	1.5	0.1649	10.05	
2.	1.5	0.1796	10.92	10.23%
Natural light 1.	1.5	0.1829	11.41	
2.	1.5	0.1815	11.03	11.22%

This table well expresses both the limitations and advantages of the chemical method of pentosan analysis. It is obvious that one cannot expect very close checking results but a valuable comparative method of testing different samples is indicated. From the results it is obvious that quite a consistent difference exists as between the pentosan content of the material grown under varied conditions of illumination. The fact that the relative percentages do not appreciably change in the plants grown for an additional twenty days is noteworthy. The explanation suggests itself that the deposition of pentosan units had reached its maximum for the conditions of the environment at the end of the 30-day period.

Again, it is indicated that the magnitude of the pentosan percentage does not directly correlate with a lowered metabolic balance, but rather seems to be related to the maturation and extent of the pentosan yielding tissues. Light is seen to be a very direct factor influencing the development of such tissues. An attempt was made to measure the light intensity received by the plants under the conditions of illumination in this experiment by the photometric method, but the apparatus was found to be quite useless under sunlight conditions.

Experiment 7.

Twelve flats of sterilized soil were prepared and planted with Marquis wheat kernels previously treated for 10 minutes in 1:1000 mercuric chloride solution. Thirty grams of an oat-hull inoculum of O. graminis were applied at seed level in ten of these flats, the two remaining flats being left as controls. Five inoculated flats

and one control were placed on a greenhouse bench and the duplicate six flats were placed beneath the bench. These flats remained in the positions indicated from January 17 to February 7, care being taken to keep the soil at the same degrees of moisture content. On February 7 the plants were dug up and notes taken on the height and infection ratings of each plant. The results appear in table 9.

Table 9. A comparison of average height and infection rating of Marquis wheat seedlings grown in natural light and subdued light in sterilized soil infested with Ophiobolus graminis.

Treatment	Number of plants	Height of plants in centimetres	Infection rating in percent
Natural light			
Inoculated	511	31.3	36.3
Control	98	37.5	0.6
Subdued light			
Inoculated	527	27.1	61.1
Control	97	35.1	1.44

Experiment 8.

In an effort to determine the degree of reliability of the above findings, another experiment was made in which measurable light sources were used and the temperature was controlled. Twelve flats of sterilized soil were planted to treated Marquis wheat, 125 seeds per flat. Ten of these were inoculated at seed level with our strain B.82 of O. graminis. Two flats were left uninoculated as controls. The twelve flats were divided into groups of six and placed in the refrigeration room in two compartments, separated by a screen. One series received the light emanating from two

200 watt Mazda lamps at a distince of 30 inches from the nearest soil surface, the other series was illuminated from a similar distance by one 100 watt Mazda lamp. The thermostatic control of the room was set at 60° F. Notes were taken on the seedlings at the end of 20 days, and the results are recorded in table 10.

Table 10. A comparison of average height and infection rating of Marquis wheat seedlings grown under controlled illumination in sterilized soil inoculated with Ophiobolus graminis.

Treatment	Number of plants	Average height of plants in centimetres	Infection rating in percent	Average soil temperature, and maximum deviation
400 Watts				
Inoculated	378	26.6	56.14	(± 18.50
Control	88	35.5	0.70	(± 1.50
100 Watts				
Inoculated	428	25.0	65.64	(± 15.50
Control	101	29.0	0.40	(± 1.50

The results of experiments 7 and 8 indicate that infection by O. graminis apparently tends to be more severe at the lower light intensities. As has already been shown, the pentosan content of wheat seedlings decreases directly with the decrease in intensity of illumination, hence some explanation other that the theory that severe infection attends an increased pentosan percentage linked with an unbalanced metabolism is needed. It is recognized that the artificial illuminants used in Experiment 8 would not be likely to promote growth at the optimum conditions of physiologic activity, yet, continuing the argument, it would be expected that the 400 watt

source would be more suitable than the 100 watt source and we should expect the metabolicequilibrium to be more seriously upset at the low light intensity. Certainly the decrease in the height of the plants would suggest this.

Experiments on the Nutrition of some Foot-rotting Pathogenes.

Experiment 9.

The first experiment under this caption was designed in an effort to determine the comparative values of the pentoses and higher sugars as carbohydrate food sources for the fungi. The pathogenes tested were: O. graminis, our laboratory strain No. 46; H. sativum, a single spore culture obtained from J. J. Christensen, Minnesota, and F. culmorum, isolated from a sample of oats from Beaverlodge, Alberta.

Thesugars used were: d-xylose, maltose, and I-Arabinose, all Pianstiehl C.P. products. These were made up in a 1.5 per cent agar medium containing in addition to 20 gms. of the sugar per litre, 2 gms. H_4NO_3, 0.5 gms. KH_2PO_4, and 0.5 gms. $MgSO_4$, all C.P. Quantities of 10 cc. were measured out separately into test tubes and sterilized at 15 pounds pressure for 35 minutes. The contents of each tube were poured into a 100 mm. Petri dish, and these plates were inoculated in the centre of the hardened medium with the organisms. The inoculum was removed with a needle point, the amounts being kept as small as possible and equal in quantity for each organism used. Inoculations were made December 11 and the plates were incubated at 25°. The first notes on the growth were taken December 14, and subsequent notes were taken at frequent

intervals thereafter until December 29. Text figure 6 indicates the progress of growth over the period of incubation.

It will be noticed from figure 6 that the disaccharide maltose provides a more utilizable carbohydrate food for all three organisms. The figures are especially significant when it is considered that the molar value of the five carbon atom sugars are to the molar value of the disaccharide as 13:.06. All media gave poor growth of H. sativum and O. graminis, the former soon exhausting the available food supply and reaching a state of inactivity. A sub-optimum food supply was thought desirable in this test in order that differences might be more marked and more readily measurable. Text figures 7 to 11 illustrate the degree and type of growth of the fungi.

No photographs could be taken of the plates inoculated with O. graminis, as the mycelium at no period showed its usual white or grey aerial growth, but rather adopted a much reduced, superficial habit.

Experiment 10. Concurrently with the above experiment a test was madeto determine the actual food value of extracts from the dried plant materials obtained from growing wheat seedlings under varied conditions of illumination. Ten gram samples of the three groups from Experiment 6 were used. To each 10-gram sample 500 cc. of distilled water were added, and the flasks autoclaved at 15 pounds pressure for 30 minutes. The flasks were now let stand for 5 hours, when the plant material was filtered off in a Buchner funnel. Two gms. NH_4NO_3, 0.5 gms. KH_2PO_4, and 0.5 gms. $MgSO_4$ were added to

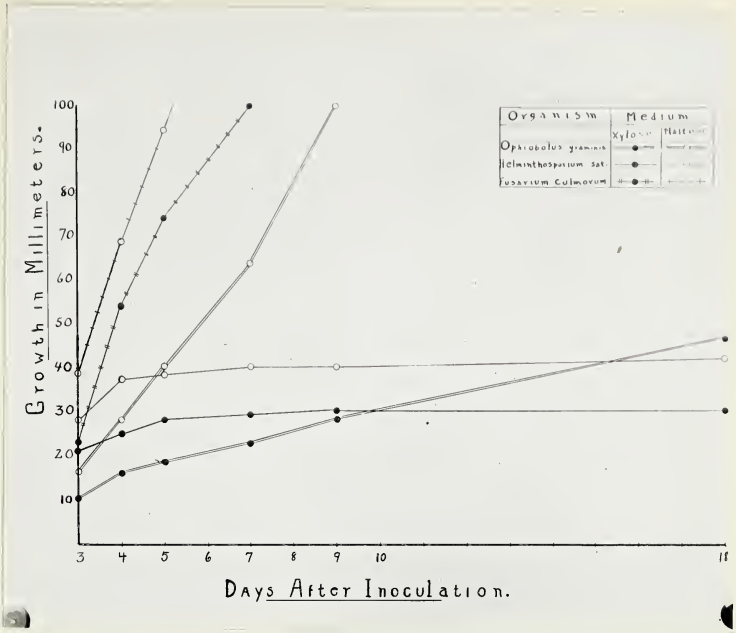

Figure 6. Graph showing growth of Ophiobolus graminis, Helminthosporium sativum and Fusarium culmorum on various sugar-agar media in 10 cm. Petri plates.

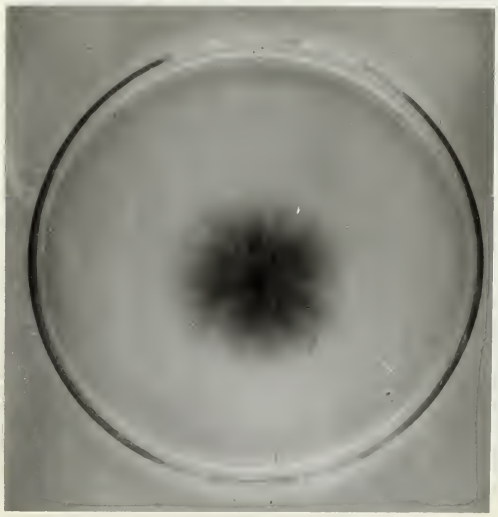

Figure 7. Four-day growth of Helminthosporium sativum on 2 per cent arabinose-agar in 10 cm. Petri plate. Note the superficial habit of the dark mycelium. X 7/10.

Figure 8. Four-day growth of Helminthosporium sativum on 2 per cent xylose-agar in 10 cm. Petri plate. Mycelium, grey, indistinct and superficial. X 7/10.

Figure 9. Four-day growth of Helminthosporium sativum on 2 per cent maltose agar in 10 cm. Petri plate. Note the aerial, white mycelium. X 7/10.

Figure 10. Four-day growth of Fusarium culmorum on 2 per cent xylose-agar in 10 cm. Petri plate. X 7/10.

Figure 11. Four-day growth of Fusarium culmorum on 2 per cent maltose-agar in 10 cm. Petri plate. X 7/10.

each filtrate and the volumes made up to 1 litre. Exact 100 cc. quantities were measured out into 200 cc. Erlenmeyer flasks, and these were autoclaved at 15 pounds for 50 minutes. After cooling, each flask was inoculated with a 5 mm. loop of an aqueous suspension of the spores of H. sativum (Christensen's strain). These flasks were incubated at 25° for 35 days. At the end of this period the resulting mycelial mats were filtered off onto tared filter papers brought to constant weight. The mycelial mats on the filter papers were dried to constant weight, and the true dry weights of the mycelia determined.

Table 11 denotes the results of this experiment.

Table 11. Weights of mycelium of Helminthosporium sativum produced on extracts from dried samples of wheat plants grown under natural, medium and low light intensities.

Light Treatment	Weights of mycelium in grams (average of 10)	Range of weights of mycelial mats in grams
Natural light	0.0575	.045 - .065
Medium light	0.0440	.035 - .045
Low light	0.0355	.030 - .040

It appears that the extract from the plants receiving the most light provides more growth promoting substances than do the extracts from plants receiving less light. It may be assumed that the energy foods supplied are of a carbohydrate nature, but just what proportions of the carbohydrate content of the respective extracts are of hexose or pentose nature have not been determined. Although the method of extraction would not be expected to exert a hydrolytic

effect, thereby freeing the pentoses from their pentosan and pectic combinations, still it seems worth while to note that the actual differences in food value of the extracts to H. sativum vary directly with the percentages of pentosan indicated by the analyses, which fact would suggest that the relative proportions of soluble and insoluble sugars vary together.

In order to determine what loss in pentosan content occurs in the plant material during the course of making an extract, duplicate samples of the plant powder remaining in the Buchner funnel were analysed in the case of the medium light material. The results showed a loss of 21.6 per cent of pentosans sustained by the treatment. This loss is probably accounted for largely by the water soluble pectic substances.

Effect of Soil Moisture on the Pentosan Content of Wheat Seedlings.

Experiment 12.

A series of sixty 1 gallon, water-tight crocks were filled

with 4 Kg. of sterilized soil. Marquis seeds, previously treated with 1/1000 $HgCl_2$ solution for 10 minutes, were planted at the 3300 gm. level at the rate of 20 per crock. The series was divided into three sections, adjusted to different moisture holding capacities by the addition of water:

A. 20 crocks at 70 per cent M.H.C.

B. 20 " " 60 " " " .

C. 20 " " 45 " " " .

The soil moisture was kept as constant as possible over a thirty-day period, when the plants were harvested. Pentosan analyses were made of the ground, dried material. The data appear in table 13.

Table 13. Dry weights and results of pentosan analyses of Marquis seedlings grown at three different soil moisture capacities.

Approximate percentage moisture holding capacity	Total dry weight of plants in grams	Weight of precipitate of phloroglucide in grams	Percentage pentosan	Total number of plants
		.0768	4.85	233
70	22.3	.0790	4.98	
		.0962	5.99	272
60	21.0	.0880	5.51	
		.1210	7.46	231
45	13.8	.1192	7.35	

From the above results one may conclude that the pentosan content varies inversely as the soil moisture. Although no test was made on a moisture holding capacity below 45 per cent, it is assumed that pentosan percentages would decrease decidedly in very

dry soils because of the abnormal plant environmental conditions. McKinney and Davis (17) observed an increase of infection by O. graminis on wheat seedlings at high soil moisture-holding capacities. They express the belief that the incidence of some soil drying agent makes disease symptoms evident upon the above-ground parts of the plants, which already were present in the root system. This statement, however, gives no information as to the actual progress of infection in dry soils, but it is thought that germination and growth of the plants under investigation will be sufficient to support the life processes of the fungus.

DISCUSSION AND CONCLUSIONS

It has been our purpose in the present study to determine what role may be played by the factor of carbohydrate metabolism in the wheat plant in the problem of disease expression as caused by the root-rotting pathogenes O. graminis, H. sativum, and F. culmorum. The necessity of investigating the reactions of these pathogenes from this angle was suggested by the work of Dickson et al (7), who have reported their conclusions regarding the relations of Gibberella saubinetii to the carbohydrate metabolism of the host plants, wheat and corn. These workers state that the pathogenicity of the organism is stimulated in hosts whose metabolic equilibrium is unbalanced, and they show that a high percentage of pentosans in the plant goes hand in hand with such an unbalanced physiological state. The factor of high soil temperature was stated to be of great influence in causing this unbalanced metabolic state in the wheat plant. However, the present investigation was designed to

study not only the effect of soil temperature, but also that of light intensity and of soil moisture, in inducing changes of the carbohydrate metabolism, the extent of such changes, and the result of them in modifying the expression of the disease in the host.

The experiments on the factor of illumination were carried out mainly in the greenhouse and in the refrigeration chamber set at suitable temperatures. Both natural sunlight and artificial illumination were used as light sources, and it was thought that the latter method, though not simulating field conditions, provided a more measurable state and made possible more accurate control of the constants, soil moisture and soil temperature. Various factors in relation to the degree of illumination received were determined, viz. the extent and amount of lignification, and of pentosan bearing tissues as demonstrated in stained free-hand sections; the pentosan percentages as determined by chemical analyses; the degree of infection of the plants by O. graminis; the value of plant extracts made from the dried plant material as food sources for the fungi in vitro; the heights of plants and their total dry weights.

Since the factor of light plays the dominant role in the processes of photosynthesis, we would expect marked differences in the carbohydrate metabolism and disease expression in the plants related to differences in the illumination received. These expectations were realized in the present work, as marked and consistent differences were apparent in the pentosan content, in the expression of disease symptoms as caused by O. graminis, and in the general

plant vigor, under different illumination intensities applied. Thus, plants growing under curtailed illumination were lower in pentosan content, were more susceptible to attack by O. graminis, and were less vigorous as expressed by their average height and the total dry weight. The stained sections showed a much less extensive lignification of the tissues and a consequent decrease in the pentosan staining area. According to the conclusions of Dickson one would expect to find such plants, obviously growing in a state of unbalanced metabolism, to show larger concentrations of the pentosan substances, but as we have not found this to be the case and have found in addition that the severity of infection by O. graminis is increased with a decrease in light intensity, one must conclude that although his theory may hold in the case of G. saubinetii and possibly with other pathogenes, it does not hold in the case of the pathogene that we have used.

The experiments on temperature effects were subjected to the degree of control obtainable in the constant temperature control tanks of the Field Crops Department. These were adjusted to a range of four values approximating 16°, 21°, 26°, and 31° C. and both summer-and winter-grown material was studied. No attempts were made to study disease expression in this case as this relation has been well covered by others (13,14,17). Only such factors as the histological observation of lignification and pentosan staining, the quantitative estimation of pentosans, and the vigor of the plants were studied. No definite difference in the pentosan percentages of plants grown at the above temperatures were noted. The tests

were not very extensive however, and it is thought that tests on more replications would be desirable. It would also be of interest, and possibly of value, to devise apparatus which would control the temperatures of the air in contact with the above ground parts of the plants in harmony with the soil temperatures, since analyses are made of the total plant tissue. There were marked differences observed in the extent of lignification and in the amount of pentosan staining tissue observed in sections from the roots of plants grown at the different temperatures, the most marked staining reaction and lignin deposition being observed in the 26° C. material. Thus, if one were basing his estimations of the relative pentosan content of the seedlings upon the observed histological findings this would prove eminently unsatisfactory in the light of the actual pentosan analyses. The chief value of the application of microchemical technique to plant sections must be mainly the orientation of the various tissue components in the structure. No definite comprehension of the plant as a living, reacting organism can be gained from such methods. On the other hand, the quantitative methods of analysis offer a clearer understanding of the general vigor and the physiological state of the plant since these characters will be expressed quantitatively in structural features and tissue organisation.

Now, considering the work of Dosdall(8), Henry (12), McKinney and Davis (17) and others who have reported upon the infective power of the foot-rot pathogens under different temperature conditions, it must be admitted that somewhat definite temperature optima exist for the various fungi. Since extremes in soil temperature may be

expected to be directly linked with extremes of soil moisture and illumination, under field conditions, it is difficult to decide just what significance to attach to this single factor of temperature. Dickson et al (6, 7, 13) have ascribed considerable importance to this factor under controlled conditions, showing that is is associated with marked differences in the degree of infection shown on wheat seedlings by G. saubinetii. They observed definite differences in the composition of cell walls and in the stability of the complexes by qualitative microchemical tests, but observed little differences in the composition of the plants grown at different temperatures by quantitative analysis for the hydrolysable sugars. This latter finding agrees with the results of pentosan analyses as carried out in the present investigation.

One experiment only on the factor of soil moisture was made, there being a series of three moisture holding capacities used of 70, 60, and 45 per cent. Notes on the plants grown included data on the pentosan content and vigor of the seedlings. Again, in this aspect of the problem it was thought that the question of infection relationships had been sufficiently covered by others to warrant omission from our considerations. Thus, our object would be attained by comparing the present data with that already published. The results showed that the pentosan content of the wheat seedlings bore a direct relation to the soil moisture conditions. It was found that these measurable factors varied inversely, but little difference in the plant vigor could be noted under the conditions of the test. However, it is thought that such manifestations of the

plants' response to its environment would have become apparent in the post-seedling stage. Since McKinney and Davis (17) have shown that infection of wheat plants by O. graminis is more severe at high soil moisture conditions, it is again seen that the parasite is favored by those plants possessing the lower pentosan percentages. Hence it must be concluded, that, aside from allegedly offering favorable and easily utilizable sources of food for the fungi, the pentosan content is but indicative of the general vigor of the host, thus any resistance noted in seedlings grown under particular environmental conditions is only indirectly related to the pentosan content and directly related to the plant vigor, of which the carbohydrate metabolism is an expression. This contention is further borne out by the results of a preliminary varietal test in which three representative wheat varieties were analysed for pentosans. It was found that the variety which appeared most resistant to the foot-rot pathogenes in the field yielded the highest percentage of pentosans. Further indication is found from the results of cultural experiments wherein H. sativum was grown on extracts of plants known to possess different percentages of pentosans, and hence different amounts of the other utilizable carbohydrates. In this case, that extract containing the greater sugar content supported the most abundant mycelial growth of the fungus tested.

Of course it must be recognized that any experiment designed to test the reactions of any particular fungus to various nutrients is complicated by the necessity of taking into consideration the

enzymatic complement of the fungus. On this account no sweeping generalizations can be applied as to the favorableness or unfavorableness of specific carbohydrates as fungous foods. Thus, it may be that pathogenes possessing alike or similar complements of enzymes will be found to show nearly similar pathogenic habits in the same plant variety.

The development of resistant wheat varieties is a most desirable method of approaching the highly intangible foot-rot problem, and it would seem, if there can be any credence attached to the results of carbohydrate metabolism studies reported in this work, that this method of approach should not be neglected. Hence, if further tests lend support to the observations already made and substantiate the hypothesis of the importance of the carbohydrate complex, it would seem a worth while task to determine by chemical analyses which of our available varieties, grown under various environmental conditions, should be included in a plant breeding program. In such a chemical study it is thought that the carbohydrates would play a major role, since the present work and others' investigations show the close relation between the expression of the carbohydrate organization and the possible resistance of the plant. Thus, aside from the question of the nutritional value of the plant sugars in vitro, the same sugars in vivo are linked up with the metabolism of the living plants and protect them by the very vigor for which they themselves are largely responsible in establishing and maintaining.

The particular carbohydrate complex of a given variety is

closely tied up with the morphological characters and ——— is subject to the laws of inheritance. Its expression, however, is dependent in great part upon the environment. Varieties possessing different inherent abilities to utilize and manifest their synthesized carbohydrates, if grown under like conditions, should exhibit a gradation of responses, or in other words, different expressions of vigor. Those varieties which succeed in maintaining a balanced carbohydrate equilibrium under extreme conditions of environment would be considered to be the most vigorous and hence the most promising parental material for a plant breeding program, provided, of course, that the vigor is associated with resistance.

SUMMARY

1. The microchemical pentosan tests are fairly reliable indices insofar as they indicate the cellular structures which contain pectic substances.
2. The results of quantitative pentosan determinations may be taken as direct indices of the extent of lignification in the wheat seedlings.
3. The extent of lignification may be correlated directly with the intensity of illumination received.
4. No significant differences in pentosan content were found in wheat seedlings grown at different temperatures, viz. approximately 16°, 21°, 26°, and 31° C.
5. The percentage infection by Ophiobolus graminis on Marquis wheat seedlings varied directly with the intensity of illumination.
6. The factor of soil moisture is thought to play a part in studies

on infection under different illumination conditions, since insufficient moisture will make above ground symptoms more manifest, whereas too abundant moisture will lead to a greater expression of the fungus activity.

7. Arabinose and xylose have been shown to be inferior to maltose as sources of carbon for Ophiobolus graminis, Helminthosporium sativum and Fusarium culmorum at the concentrations tested.

8. Extracts made from plant material bearing a high percentage of pentosans induced a more copious growth of the mycelium of Helminthosporium sativum than did extracts from plant material containing less pentosan.

9. Carbohydrate sources other than pentosans provided the main carbon nutrient in these extracts.

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